TOXICITY AND ANTI-NUTRITIONAL FACTORS IN *Delonix regia* SEEDS

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ABSTRACT

The toxicity and anti-nutrition properties of Delonix regia were determined using nine rabbits to determine the median Lethal Dose (LD_{50}). The pods of Delonix regia were collected from 30 trees at Ahmadu Bello University Zaria and soaked in body of water for the pods to split. The seeds were sun-dried and grounded in hammer mill. To determine the toxicity, methanolic extract of the plant was assayed on experimental rabbits. It was observed that there was no sign of drowsiness, depression and death on the nine rabbits studied at 1000/100ml per body weight. Although, the extract could kill the test population by using median lethal dose (LD_{50}), 1000/100ml of the concentrate could not kill 50 % of rabbits in the test populations. This implies that Delonix regia seeds are not toxic but safe for inclusion into rabbits and mono-gastric diets. However it is recommended that the seeds should be cooked at 100^{o}C for 60 minutes to destroy all anti-nutritional factors to a threshold level. Farmers are encouraged to use Delonix regia seeds for mono-gastric animal diets.

INTRODUCTION

Seeds often contain factors which are deleterious or indeed toxic to animals or man (Liener, 1996). There is a wide distribution of biologically active constituents throughout the plant kingdom, particularly in plants used as animal feeding stuff and in human nutrition (Igile, 1996). The knowledge that these compounds elicit both toxic and advantageous biological response has given rise to several investigations in recent times as to their possible physiological implications in various biological systems (Igile, 1996). These anti-nutritional factors need to be removed or inactivated by extensive predetermined heat treatment of seed diet. However, effective pre-treatment is difficult to achieve if there is limited availability of fuel for cooking. This is particularly a serious problem in relation to the phytate and other factors since they are quite resistant to heat treatment. Anti-nutritional factors diminish animal productivity but may also cause toxicity during periods of scarcity confinement when the feed rich in these substances is consumed by animals in large quantities (Kumar, 1992). The cyanogenic glucose on hydrolysis yields toxic hydrocyanic acid (HCN). The cyanide ion inhibits several enzymes; depress growth through interference with certain nutrients. They also cause acute toxicity, neuropathy and death (Frenando, 1987). Alkaloid cause gastrointestinal and neurological disorder (Aletor, 1993). Tannin causes decreased feed consumption in animals, binds dietary protein and digestive enzymes to form complexes that are not readily digestible (Aletor, 1993). They also cause decreased palatability and reduced growth rate (Roeder, 1995). Trypin (Protese inhibitor causes pancreatic enlargement and growth depression (Aletor and Fetuga, 1987). Substances which occur naturally in food manifest their toxicities especially when consumed with food in large or little doses. Some of these dietary or anti-nutritional factors interfere with bioavailability of nutrients and constitute a major factor

limiting the wider food use of such tropical plants. The presence of phytate in foods is known to lower the bioavailability of minerals and inhibits several proteolytic enzymes and amylase (Singh *et al*., 1996). It is therefore essential that any potential food sources should be examined for anti-nutritional factors and toxicity. A general survey of anti-nutritional properties of several tropical seeds are been considered for future development is been undertaken on *Delonix regia* seeds.

MATERIALS AND METHOD

Toxicity Assay

The toxicity of the extract was measured by the method described by Lorke (1983) *Delonix regia* seeds were oven dried and ground into fine powder. The powdered flour was put in an extraction funnel and methanol was added. This was allowed to stand for 24 hours for the extraction of the seed concentrate to take place (cool extraction). The concentration was allowed to stand for several days until the methanol evaporated leaving only the seed concentrate. Care was taken to ensure that no trace of the methanol was found in the concentrate. Precisely 2.5gram of the extract was dissolved in 3ml of pure vegetable oil which was sealed before used to feed the rabbits kept in three groups of three animals each. The test rabbits were of the same weight and age. The extract was thereafter made up to 500g and used for toxicity measurement.

The extract was administered to three groups of rabbits of different weights and was observed 24 hours for signs of drowsiness, depression and death.

Analysis of Anti-nutritional Properties

(a) Determination of Trypsin Inhibitor Activity (TIA)

Trypsin inhibitor activity of sample was determined by the method of Kakade *et al* (1974). The digest contained 1.0g of the sample, 40g of trypsin and 2.0mg of Benzoyl-DL-argine-P-nitroanilde (BAPA) in tris buffer. The absorbance of sample was read at 410nm.

(b) Determination of Cyanogenic Glycoside Content

Cyanogenic glycoside contents of ample were determined by alkaline titration method Wheeler and Ferret (1971). Briefly, samples (1.0g each in triplicate) dissolved in 200ml distilled water were distilled for 2hrs to collect 150cm^3 of distillate. To the distillate, was added 20cm^3 of a 2.5% NaOH and the volume made up to 250cm^3. To samples (100cm^3 of diluted distillate) was added 8.0cm^3 of 6M NH_4OH solution and 2.0cm^3 of 5% KI, and then titrated against 0.02M $AgNO_3$ solution using 10cm^3 micro burette. The end-point was noted as a permanent turbidity against a black background. Titre values were obtained and cyanogenic glycoside contents calculated using the formula.

$$\text{Cyanogenic glycoside mg/100g} = \frac{\text{TV x 1.08 x EV X 100}}{\text{SM x AL}}$$

Where:

TV = Titre Value (cm^3)
EV = Extract Vol. (cm^3)
SM = Sample Mass (g)
AL = Aliqout (cm^3) useds
N/B 1 cm^3 of 0.02N $AgNo_3$ = 1.08mg HCN.

Tannin contents of samples were determined by the method of Folin-Dennis as described by Pearson (Pearson, 1976).

Determination of Phytic Acid:
An indirect colorimetric method of Wheeler and Ferrel (1971) was used for phytate determination. This method depends on an iron to phosphorus ratio of 4:6. Five grams of the test sample was extracted with 3% tri-chloro acetic acid. The phytate was precipitated as ferric phytate and converted sodium hydroxide. The precipitate was dissolved in hot 3.2N HNO and the colour read immediately at 480nm. The standard solution was prepared from Fe $(NO_3)_3$ standard curves. The phytate concentration was calculated from the iron results assuming a 4:6 iron: phosphorus molecular ratio.

Statistical Analysis:
The data obtained in this work were subjected to statistical analysis using statistical programmes in Microsoft Excel and Statistical Package for the Social Sciences (SPSS 10.0 package). The statistical analysis carried out was mean and standard deviation, analysis of variance (ANOVA). Duncan's Multiple Range (DMR) test (Alder and Roessler, 1977; Ogbeibu, 2005).

RESULTS AND DISCUSSION

The acute toxicity of *Delonix regia* seeds is presented in Table 1. The acute toxicity carried out on rabbits shows that there were no signs of drowsiness and depression. Similar results have been obtained by Vaishali *et al*, (2012), on the extract of the leaves of *Delonix regia* seeds (150-200g) administered to Wister albino rats and mice (20 – 25g) of either sex *Delonix regia* seeds, according to these authors, was safe up to a dose level of 5000mg/kg of body weight and no lethality or any toxic reactions were found up to the end of their study period.

The absence of death in the 3 group's o rabbits treated with *Delonix regia* seeds concentrate as high as 1000ml/100ml of the extract indicated a high margin of safety (Lorke, 1983). The seeds of *Delonix regia* are safe for inclusion into rabbits and monogastric diets since 1000ml/100ml of the concentrate could not kill 50% of the test population LD(50). It was not possible to determine the LD(50) of the extract because high volumes of it would have been administered and could result into death due to volume effect rather than a toxic effect of the extract. This unaltered toxicological profile indicates that the extract was free of any side effect.

The results of anti-nutritional factors are presented in Table 2. The raw *Delonix regia* seeds contain high levels of anti-nutritional factors. There was a significant (P>0.05) reduction on the levels of these factors as the duration of cooking increased. The most affected was trypsin inhibitor activity, followed by tannin, phytate and hydrocyanic acid. Boiling for 60 minutes at 100^{o}C was able to reduce trypsin inhibitor, phytate, tannin and hydrocyanic respectively to a threshold level.
The result clearly demonstrated that trypsin inhibitor had higher susceptibility to heat than other anti-nutritional factors commonly called secondary metabolites. This confirmed an earlier observation by Wanjekechi *et al*. , 2003; Bawa 2003 and Abdullahi and Abdullahi, 2005).

Table 1: Acute Toxicity of Graded Levels of *Delonix regia* Seeds Concentrate

Dosage per 100ml	**Weight of Rabbits (g)**			**No. of Deaths**
	800g	400-600g	300-400g	
10				0
100				0
1000				0

Table 2: Anti-nutritional Factors in *Delonix regia* Seeds Cooked in 100°c for varying period.

Parameter	0 min.	15min.	30min.	45min.	60min.	75min	90min	SEM	LOS
Trypsin Inhibitor (mg/100g)	9.23[a]	6.08[b]	3.15[c]	2.00[d]	1.98[d]	1.89[d]	1.81[d]	0.11[b]	*
% Destruction	0	34.13	65.59	78.33	78.54	79.52	80.39		
Phytic Acid (mg/100g)	8.27[c]	6.95[b]	4.75[c]	4.16[d]	4.07[ed]	3.97[e]	3.77[e]	0.106	*
% Destruction	0	15.96	42.56	49.70	50.79	52.00	54.44		
Tannin (mg/100g)	0.43[a]	0.31[b]	0.20[c]	0.17[c]	0.16[c]	0.14[c]	0.11[d]	0.013	
% Destruction	0	27.91	53.49	60.47	62.79	67.44	74.42		
Cyanide (mg/100g)	2.06	1.70[a]	1.56[b]	1.29[c]	0.64[d]	0.60[d]	0.57[d]	0.0665	*
% Destruction	0	17.48	24.27	37.38	68.93	70.87	72.33		

Figures followed by the same letter(s) in each row are not significantly different (P<0.05) using DMRT.

SEM: Standard Error of Means
LOS: Level of Significance
***:** Significant (P 0<0.05)
NS: Non-significant difference

CONCLUSION AND RECOMMENDATION

The result of this study indicates that *Delonix regia* seeds are not toxic since there was no depression, drowsiness and death recorded. The seeds are safe for inclusion into rabbit's diets since 1000/100ml of extract could not kill the test population (LD_{50}).

It is therefore, recommended that the seeds should be cooked for 60 minutes at 100°C to reduce all anti-nutritional factors in the seeds. Effort should be geared towards finding other means of processing the seeds in order to render them more useful and useable as protein source for monogastric animals. Farmers are encouraged to use *Delonix regia* ratio formulation.

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